

1. PURPOSE

This procedure describes how to synthesize cDNA from RNA using the ThermoScript RT-PCR Kit.

2. SCOPE

The instructions are for synthesizing cDNA from RNA samples to be used for QGE (Quantitative Gene Expression) application service.

3. REFERENCES

- 3.1. *ThermoScript™ RT-PCR System*, Part n. 11146.pps, Rev. date: 26 Sep 2003, *Invitrogen*.
- 3.2. Application note: *Multiplexed Gene Expression Analysis Using Competitive PCR and MassARRAY (Sequenom Inc)*.

4. EQUIPMENT, REAGENTS, AND MATERIALS

- 4.1. Thermal Cycler
- 4.2. Tube Vortexer
- 4.3. Tube Centrifuge
- 4.4. ThermoScript™ RT-PCR System Kit, *Invitrogen*
- 4.5. 0.5 mL micro tubes
- 4.6. 200 uL pipette, 20 uL pipette, and 2.5 uL pipette
- 4.7. 200 uL and 10 uL Pipette tips
- 4.8. Trough

5. RESPONSIBILITIES

It is the responsibility of trained personnel synthesizing cDNA according to the guidelines described in this procedure.

6. PROCEDURES

6.1 cDNA Synthesis

- 6.1.1 In a 0.5-mL tube, combine 50 ng/uL random primer, approximately 1 ug RNA, dNTP mix, and adjust volume to 12 uL with DEPC-treated water for each sample.
- 6.1.2 Vortex and centrifuge tubes.
- 6.1.3 Denature RNA and primer contained in tubes by incubating at 65°C for 5 minutes.

Program ID: DENATURE

PCR Cycle	Cycling Conditions
Incubation	65°C for 5 minutes
Hold	4°C

- 6.1.4 Vortex the 5X cDNA Synthesis Buffer for 5 seconds just prior to use.
- 6.1.5 Prepare a master reaction mix for total number of sample reactions plus at least 25% using the values shown in Table below.

Reagents	Concentration	8 uL Conc.	1 Rxn
5x cDNA Synthesis Buffer		N/A	4
DTT	0.1 M	12.5 mM	1
RNaseOUT	40 U/uL	40 U	1
DEPC-treated water		N/A	1
ThermoScript RT	15 U/uL	15 U	1
Total Volume	uL		8 uL
Dead Volume	20%		

- 6.1.6 Vortex and centrifuge master reaction mix.
- 6.1.7 Add 8 uL of master reaction mix into each reaction tube.
- 6.1.8 Vortex and centrifuge reaction tubes.
- 6.1.9 Transfer the sample to a thermal cycler and incubate as shown below:

Program ID: RH

PCR Cycle	Cycling Conditions
Initial Incubation	25°C for 10 minutes
Secondary Incubation	50°C for 50 minutes
Terminal Incubation	85°C for 5 minutes
Hold	4°C

- 6.1.10 Add 1 uL of RNase H to each reaction tube.
- 6.1.11 Transfer reaction tubes to thermal cycler and incubate at 37°C for 20 minutes.

Program ID: 37-20M

PCR Cycle	Cycling Conditions
Incubation	37°C for 20 minutes
Hold	4°C

- 6.1.12 Store cDNA synthesis reaction tubes at -20°C for daily use or at -80°C for long term storage.

7 RECORDS REQUIRED FOR THIS SOP

8 ATTACHMENTS

9 HISTORY OF REVISION

<u>REV NO.</u>	<u>EFFECTIVE DATE</u>	<u>PAGES REVISED</u>	<u>CR NUMBER</u>
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